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ROLE OF THE KIDNEY IN ELIMINATING PROTEINS AND PEPTIDES

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INTRODUCTION

The kidney plays several roles in the elimination of proteins and peptides. Proteins that are too large to be filtered at the glomerulus must be eliminated by other routes. In contrast, proteins or peptides of molecular size and charge to allow filtration at the glomerulus have appreciable elimination by the kidney; in fact, this elimination may be so rapid as to preclude clinical benefit of the protein. The filtration barrier at the glomerulus restricts proteins of molecular size > 40Å. In addition, the glomerular filtration barrier has a net negative charge. Thus, proteins somewhat smaller than the glomerular pores may still have restricted filtration if they also have a net negative charge. Proteins substantially smaller than the molecular pores are freely filtered even if their charge is negative. The importance of glomerular filtration of recombinant proteins is best illustrated by small molecules that are eliminated so quickly that a therapeutic effect is likely precluded. Examples include α_1-proteinase inhibitor and superoxide dismutase (SOD). Because of the rapid elimination of these two compounds, modifications have been made to the molecule in order to sustain them in plasma for longer periods of time while maintaining pharmacologic activity. Methods for accomplishing this goal will be discussed.

Once proteins and peptides are filtered, catabolism occurs by peptidases at the brush order of the proximal nephron. The resultant small peptides and amino acids are then reabsorbed by the proximal tubule. This reabsorption contributes to isotonic reabsorption of water at the proximal nephron. Two characteristics of this catabolism are important when considering the kidney's role in eliminating proteins and peptides. First, catabolism is saturable as has been demonstrated with SOD. Secondly, this catabolism is inhibitable and so doing allows expression of pharmacologic effects at more distal sites in the

nephron. Examples of this phenomenon include atrial naturietic peptide (ANP) and the antibiotic, imipenem.

GLOMERULAR FILTRATION OF PROTEINS AND PEPTIDES

α_1-Proteinase Inhibitor

Native α_1-proteinase inhibitor is primarily eliminated by non-renal routes. However, recombinant α_1-proteinase inhibitor (rα_1-PI) derived from yeast is not glycosylated and this form of the enzyme is eliminated by the kidney. In addition, such elimination is so rapid as to preclude therapeutic effectiveness. In order to restrict glomerular filtration, rα_1-PI has been conjugated to polyethylene glycol. Polyethylene glycol (PEG) can be synthesized with a variety of sizes and when coupled with a protein can increase the overall molecular size sufficient to impair glomerular filtration and allow a more prolonged presence in plasma. Table 1 shows data demonstrating this phenomenon with rα_1PI in mice (1):

TABLE 1: Renal Elimination of Recombinant α_1-Proteinase Inhibitor (from reference #1)

	Half-Life (min)
rα_1PI	12
+ Renal Ablation	>60
rα_1PI – PEG-2 (M_r = 2000)	110
rα_1PI – PEG-4 (M_r = 4000)	>600

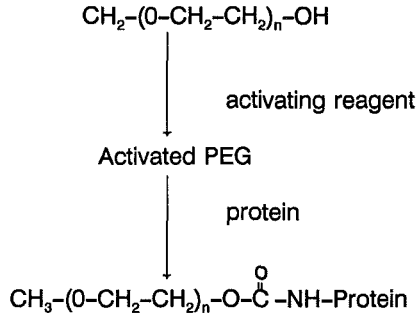
The half-life of rα_1PI in healthy mice is approximately 12 min. The dramatic increase in half-life with renal ablation indicates that elimination is predominately via renal mechanisms. Coupling rα_1PI to PEG having a molecular radius of 2000 (PEG-2) increases the half-life to 110 minutes whereas coupling of the protein to a higher molecular weight form of polyethylene glycol increases the half-life even more (1). Importantly, the pharmacological activity of rα_1PI is retained even when coupled to PEG. This strategy of coupling PEG to a protein to increase its molecular size can also be used with other proteins since the

conjugation procedure is general (2). Table 2 shows some of the activating reagents that have been used for coupling PEG to proteins and indicates a schematic for the reaction. It is important to note that the coupling reaction may interfere with the pharmacologic activity of the protein itself and that this may differ depending upon the activating reagent.

TABLE 2: Polyethylene Glycol (PEG) Conjugation with Proteins

Couples PEG to the ε-amino group of proteins by activating the hydroxyl group on PEG.

1. Activating reagents
   a. Cyanuric chloride
   b. 1,1'-carbonyldiimidazole
2. Synthesis



Note: Coupling may impair pharmacologic activity.

Superoxide Dismutase

As indicated above, coupling of PEG to rα_1PI results in retention of pharmacologically active drug in the plasma for a longer period of time. This mechanism has also been used to retain SOD in plasma (2). As shown in Table 3, the half-life of SOD in experimental animals is several minutes, whereas renal ablation extends this half-life considerably. For example, in healthy rats the half-life of SOD is 6 minutes, whereas renal ablation increases this value to 55 minutes, indicating that SOD depends upon the kidney for elimination. This

dependence upon renal elimination has also been confirmed by micropuncture techniques and autoradiographic localization of SOD to the proximal tubule (3). SOD can also be coupled to PEG without losing its pharmacologic activity and so doing dramatically increases the elimination half-life as has been demonstrated in mice (2).

TABLE 3: Half-Life of SOD (M_r = 25Å) vs PEG-Modified SOD

	SOD		SOD-PEG
	Normal Renal Function	Renal Ablation	
Rat	6.0±0.5 min	55±5 min	
Mouse	3.5 min		9 hr 16.5 hr
Man	1.4-3.3 hrs		

Other similar methods have also been used to preclude SOD from glomerular filtration. Molecular engineering techniques have been used to couple two SOD molecules together via a spanning segment (4). So doing increases the molecular size sufficient to prevent renal elimination while retaining pharmacologic activity. Alternatively, SOD has been conjugated to albumin which also accomplishes the same goals (5).

In summary, rapid glomerular filtration of small proteins and peptides may frequently preclude their utility as therapeutic agents. One viable mechanism for restricting glomerular filtration and renal elimination, thereby retaining them in plasma for enough time to exert clinically relevant pharmacologic activity is to derivatize the protein. This can be done in a fashion that will increase the protein's size sufficient to restrict its filtration. Successful employment of this technique has been demonstrated with α_1-proteinase inhibitor and with SOD.

This can be accomplished with polyethylene glycol and other conjugates; it is a promising technique for use with other proteins.

CATABOLISM OF FILTERED PROTEINS

Saturation

Once proteins are filtered by the glomerulus, they are catabolized by peptidases associated with the brush border of the proximal nephron. Amino acids and small peptide fragments are then reabsorbed by the proximal tubule. This catabolic capacity is saturable as has recently been demonstrated with SOD (Brater and Odlind, unpublished data). Studies in animals have shown that elimination of SOD is exclusively by the kidney (3). However, at low doses, only very small amounts, if any, SOD appear in the voided urine. With increasing doses, SOD escapes catabolism at proximal nephron sites and is detected in the urine. This phenomenon creates an interesting anomaly in which total clearance of SOD in man would ordinarily be considered to be via non-renal clearance. This is because the renal clearance is calculated to be negligible since little, if any, drug appears in the urine. This observation is in contrast to the fact that renal elimination is known to be the sole pathway for SOD excretion (3). For example, in healthy volunteers with a dose of 1 mg/kg, the total clearance of SOD is approximately 4L/hr, and based on amounts of SOD appearing in the urine, renal clearance only accounts for 2.6% of total clearance. With increasing doses of SOD and as catabolism in the proximal nephron is saturated, more drug reaches the urine and it thereby appears that a greater proportion of clearance is by renal routes. For example, with a dose of 15 mg/kg, the total clearance of SOD in normal volunteers remains at approximately 4L/hr, but at this dose 52% of clearance can be accounted for by SOD appearing in the urine. In this circumstance, calculated renal clearance is not a valid reflection of the renal contribution to elimination of SOD, but instead represents a mechanism for quantifying the saturability of catabolism of SOD at the proximal nephron.

Inhibition

In addition to being saturable, it is also clear that catabolism of at least some proteins and peptides at the level of the proximal nephron can be

inhibited. This has been demonstrated to occur with atrial natriuretic peptide (ANP) (6). Filtered ANP is catabolized by neutral endopeptidase at the brush border of the proximal nephron. As a consequence, ANP's effects at more distal nephron sites are precluded. Recent studies have shown that inhibition of neutral endopeptidase results in pharmacologic effects of ANP more distally (6). As such, increases in urinary volume, sodium, and fractional excretion occur, which correlate with increases in fractional excretion of ANP itself and increases in urinary cyclic GMP, the second messenger for ANP effects:

TABLE 4: Inhibition of ANP Catabolism by the Proximal Nephron* (from reference #6)

	Control	ANP Alone	ANP Plus NEP Inhibition
Volume (ml/min)	0.25	1.18	2.94
Sodium (μEq/min)	52	199	410
FE_{Na} (%)	1.2	4.0	7.5
cGMP (pmol/min)	624	724	1404

*ANP = Atrial Natriuretic Peptide
NEP = Neutral Endopeptidase
FE = Fractional Excretion
cGMP = Cyclic Guanosine Monophosphate

Data in Table 4 reveal that ANP alone causes increases in sodium and volume excretion but these increments are dramatically increased when ANP catabolism is prevented by inhibition of neutral endopeptidase. Moreover, these

increases occur in concert with increases in fractional excretion of ANP and in excretion of cyclic GMP, offering strong evidence that the effect is due to increased delivery of ANP to more distal sites of the nephron where it exerts a pharmacologic effect.

Though imipenem is not a peptide or protein, it is a good illustration of the potential utility of inhibiting catabolism of a compound at proximal nephron sites. Imipenem is both filtered and secreted by the kidney but it is then catabolized by a dipeptidase at the brush border of the proximal tubule. This process affects both filtered and secretory components of imipenem that appear in the nephron. This catabolism precludes any antibacterial affect of this drug at more distal sites of the kidney or in the bladder. Thus, imipenem by itself is not effective for urinary tract infections. However, if imipenem is administered with an inhibitor of dipeptidase, the amount of imipenem appearing in the urine increases from about 5% to as much as 70% of the administered dose which is sufficient to have therapeutic activity in the urine (7). The dipeptidase inhibitor used with imipenem is cilastatin, which was chosen among several possibilities simply because its pharmacokinetic profile parallels that of imipenem itself.

Data with ANP and with imipenem illustrate that inhibition of catabolism of compounds and proteins can result in a pharmacologic effect at more distal sites in the nephron. The potential clinical utility of this phenomenon with imipenem is obvious in that it allows use of this drug for treatment of urinary tract infections. The utility of such an approach with ANP has not been determined through clinical studies but might be important in enhancing the effects of ANP in clinical conditions in which circulating concentrations of ANP are elevated (for example, congestive heart failure), but in which little natriuretic effect occurs that can be attributed to ANP. Inhibition of its catabolism at the proximal nephron might allow endogenous amounts of ANP to exert a natriuretic effect and therefore be beneficial in the clinical conditions in which ANP itself is elevated. Whether or not such a strategy could be clinically useful awaits further studies.

CONCLUSION

The kidney plays vital roles in the elimination of proteins and peptides. Understanding these roles allows their manipulation in a fashion that will allow use of proteins and peptides more effectively as therapeutic agents. Promising advances have been made in this field and it is anticipated that more will follow.

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Discussion : THE ROLE OF THE KIDNEY IN ELIMINATING PROTEINS AND PEPTIDES

H.J. Röthig

Does anyone know of small peptides which may be secreted by the tubular system so that renal excretion exceeds glomerular filtration?

P. du Souich

Argine vasopressin is filtrated and also secreted into the tubule, and in fact this secretion can be inhibited.

R.G. Werner

Is there any influence of the polyethylene glycol coupling on the activity of the peptide? And, what about stability and shelf life?

D.C. Brater

As far as the loss of activity is concerned, this is highly variable, and it has been shown that there are molecules where the coupling of polyethylene glycol eliminates all activity, while in others virtually none is lost, and in some the loss is intermediate. So in many cases enough activity seems to be preserved as to make them potentially therapeutically useful. On the other hand I do not know about shelf stability of a modified peptide.

M.M. Reidenberg

Is the kidney an important eliminating organ for fragments of some of these proteins that have been partially hydrolyzed, and if so might these accumulate in people with poor kidney function?

D.C. Brater

I would assume that the kidney is certainly an important organ for the elimination of these fragments. If one does not see accumulation when this process of elimination is not functioning one has to postulate the existence of alternative pathways, which are probably going to be highly variable. But I would think that one would certainly have to worry about them.

D. Maruhn

In the case of alpha 1 proteinase inhibitor we should not forget that there is an alternative, that is to produce this protein in a glycosilated form from plasma which has considerable half life and is probably acceptable for therapeutic purposes. On the other hand, as far as the saturable catabolic process in the kidney is concerned, it could be that the lysosomal capacity in the proximal tubule is what is really saturated.

D.C. Brater

In the SOD studies we also monitored some of the proximal tubule enzymes which I believe are lysosomal. And I would presume that if we were overloading the lysosomes we would have seen an increase in the levels of those enzymes in the urine, but we saw no difference in enzymuria with 45 mg/kg as opposed to 1 mg/kg. I would think that this data might be some indirect evidence that what we are really doing is overwhelming the peptidase rather than the lysosomal capacity.

J.A. Galloway

One possible example of your model is our finding that although human proinsulin is disposed of primarily in the kidney, and the half-life in patients with renal failure is prolonged, virtually no proinsulin is found in the urine.

P. du Souich

I imagine that accumulation of peptides in patients with renal failure is unlikely, in view of the large amounts of endopeptidases in other tissues, such as the lung or the intestines. Also, I have a question concerning SOD. Since it exerts its scavenger effect in the cytosol usually, how the binding to a large molecule is going to affect the entry of the superoxide dismutase into the cell?

D.C. Brater

As far as I know, in an animal model of cardiac ischemia and reperfusion injury the coupled SOD does seem to get to where it needs to go. This is just indirect proof, and of course efficacy may be by a different mechanism, but I think yours is a very good question.

L. Gauci

Since we are talking about pharmacokinetics I think that one of the major differences between protein drug development and conventional drug development is the

rendering of plasma kinetics almost useless in the former.

M.M. Reidenberg

I think that too often kinetics studies are viewed as the end rather than the means. I think we need to differentiate the kinetics of the molecule from the kinetics of the effect. And that information on the kinetics of drug effect rather than just the disappearance rate of the compound probably can be useful in developing dosage regimens. Bob Meyer gave an example where kinetics of the molecule itself is important with respect to the releasing factors and whether one wants a pulsatile pattern or a steady-state pattern. So I think the issue of kinetics like any other scientific methodology is related to what is the question that the methodology is intended to answer rather than saying the methodology is an end into itself. The methodology development was an end several decades ago when the whole field of pharmacokinetics itself was being developed but now that the methods exist it is up to us to use them to answer questions rather than as ends in themselves.